

Addition to the known diversity of Chinese freshwater mussels: integrative description of a new species of *Postolata* Dai et al., 2023 (Bivalvia, Unionidae, Gonideinae)

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Abstract

In this study, we present a new species of freshwater mussel in the genus *Postolata* Dai et al., 2023, from Guangxi Province, China, by integrating morphological, anatomical, and molecular data. *Postolata longjiangensis* Liu & Wu, **sp. nov.** is distinguished from its congener (i.e., *Postolata guangxiensis*) by its shell shape, beak position, surface sculpture, nacre color, and hinge structure. Molecular species delimitation results based on the mitochondrial COI gene support the separation of *Postolata longjiangensis* Liu & Wu, **sp. nov.** from its congener. The multi-locus (COI + 16S rRNA + 28S rRNA) phylogeny reveals that this species forms the sister lineage to *Postolata guangxiensis* in the tribe Gonideini.

Key Words

China, cryptic species, freshwater mussels, integrative taxonomy, multi-locus phylogeny, *Postolata*

Introduction

Freshwater mussels (order Unionida) are renowned for their distinctive life cycle, characterized by a parasitic phase primarily reliant on fish hosts and an uncommon doubly mitochondrial inheritance (Barnhart et al. 2008; Modesto et al. 2018; Guerra et al. 2019). They are globally distributed in freshwater habitats, with the highest levels of diversity observed in East Asia and North America (Zieritz et al. 2018; Graf and Cummings 2023). This group plays crucial ecological roles, with certain mussel species simultaneously fulfilling the criteria of indicator, umbrella, and flagship species (Howard and Cuffey 2006; Vaughn et al. 2008; Vaughn 2018). Unfortunately, freshwater mussels have become one of the most imperiled faunas worldwide, with an increasing number of species at risk of extinction (Lydeard et al. 2004; Haag and Williams 2014; Ferreira-Rodríguez et al. 2019).

Consequently, the urgency of describing their diversity and systematics is highlighted by the growing research efforts and conservation attention dedicated to this group (Lopes-Lima et al. 2017a, 2017b; Do et al. 2018; Huang et al. 2019; Liu et al. 2022).

The Guangxi Zhuang Autonomous Region (hereinafter referred to as Guangxi), located in southern China and sharing a border with Vietnam, plays a significant role within the Indo-Burma biodiversity hotspot situated in the Chinese region (Tordoff et al. 2012). In recent years, new species of freshwater mussels such as *Postolata guangxiensis* Dai et al., 2023, *Pseudocuneopsis yangshuoensis* Wu & Liu, 2023 and *Pseudocuneopsis wuana* Liu & Wu, 2023 have been discovered in Guangxi (Dai et al. 2023; Wu et al. 2023b; Liu et al. 2023). It suggests that the unique habitats and geographical flora of this region may harbor previously undescribed species, while still underestimating the level of species diversity.

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The freshwater mussel genus *Postolata* Dai et al., 2023 belongs to the tribe Gonideini in the subfamily Gonideinae (Dai et al. 2023; Wu et al. 2024). It was recently established as a monotypic genus comprising only one species, namely *Postolata guangxiensis* Dai et al., 2023, which is endemic to Guangxi province in China.

In this study, another new species of *Postolata*, also from Guangxi, is diagnosed and described. We employ an integrative taxonomic approach that incorporates morphological, anatomical, and molecular phylogeny to identify and differentiate this species.

Materials and methods

Specimen collection, identification, and anatomical observations

In April 2024, six freshwater mussel specimens were collected from a rural streamlet at an altitude of approximately 150.18 m in Hechi City, Guangxi Province, China (24.530716°N, 108.5762°E; Fig. 1). Meanwhile, two specimens of *Postolata guangxiensis* were collected from the type locality (Luoqing River, Guangxi Province; Fig. 1). All specimens were deposited as vouchers at the Museum of Zoology, Shanxi Normal University (SXNU), China (voucher numbers SXNU_24040701–SXNU_24040706 for *Postolata longjiangensis* sp. nov.; voucher numbers SXNU_PG_22102301 and SXNU_PG_22102303 for *Postolata guangxiensis*).

The conchological and anatomical features of all individuals were visually examined with the naked eye and under a stereoscopic microscope (CX31-12C03, Olympus Corporation, Japan), including shell shape, umbo position and sculpture, shell surface sculpture, hinge structure, muscle attachment, and papillae in the incurrent and excurrent apertures (Figs 2, 3). The anatomical features of the soft body were described according to Williams et al. (2008).

DNA extraction, amplification, and sequencing

According to the manufacturer's instructions, a small piece of foot tissue was excised for DNA extraction using the TIANamp Marine Animals DNA Kit (Tiangen Biotech, Beijing, China). Three gene fragments, i.e., the mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA), and the nuclear gene of 28S ribosomal RNA (28S rRNA), were sequenced based on our previous studies (Wu et al. 2024). PCR amplification was implemented in a 25-μL volume using the following thermal cycling conditions: 3.5 min at 94 °C, followed by 35 cycles of 94 °C for 30 sec, 50 °C for 30 sec, 72 °C for 1 min, and a final extension of 72 °C for 5 min. The amplified PCR products were purified and sequenced by Sangon Biotech (Shanghai). All newly obtained sequences in this study have been submitted to GenBank.

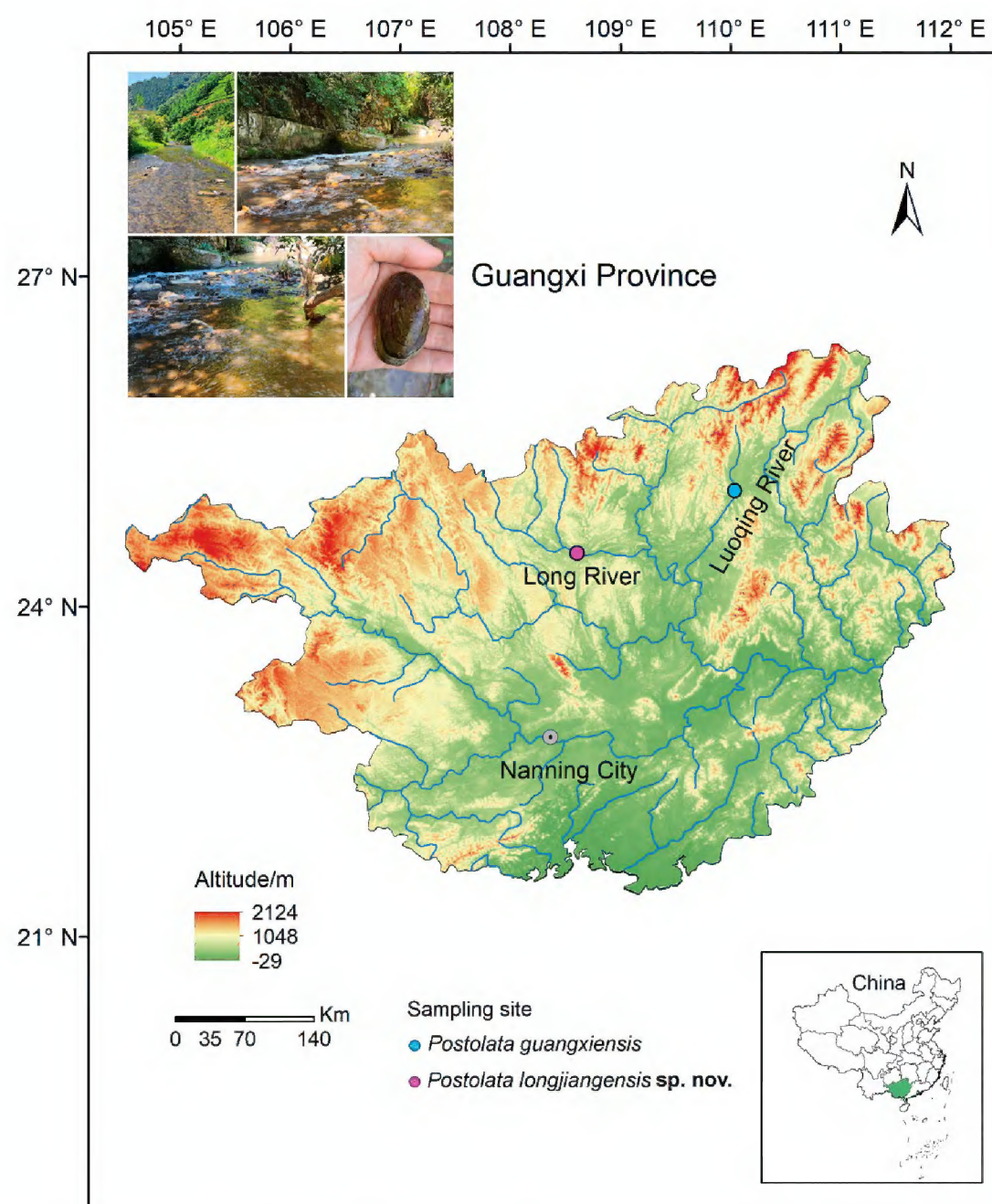


Figure 1. Map (Guangxi Province) of sampling localities of *Postolata* species and habitat of *Postolata longjiangensis* sp. nov.

Alignments, partitioning strategies, and model selection

In this study, we constructed two datasets. First, a DNA barcoding (COI) dataset for molecular species delimitation was compiled (Table 1). We downloaded the COI sequences of eight species in the tribe Gonideini, as well as all published COI sequences of *Postolata guangxiensis*, along with COI sequences from the new species described in this study. Two species from the tribe Lamprotulini, i.e., *Lamprotula caveata* (Heude, 1877) and *Lamprotula leaii* (Gray, 1833), were selected as outgroups. Second, a three-gene dataset for phylogenetic analysis was prepared (Table 2). This dataset contains species from all eight recognized tribes in the subfamily Gonideinae. In addition, *Margaritifera dahurica* (Middendorff, 1850) and *M. margaritifera* (Linnaeus, 1758) from the family Margaritiferidae were selected as outgroups.

The molecular data analyses and phylogenetic reconstruction were consistent with the methods used in our previous studies (Wu et al. 2023a; Wu et al. 2024). Protein-coding genes (COI) were aligned by built-in MACSE with invertebrate mitochondrial codon modes implemented in PhyloSuite v1.2.3 (Zhang et al. 2020). Ribosomal genes (16S rRNA and 28S rRNA) were aligned using MAFFT v7.2 (Katoh and Standley 2013) with the L-INS-i algorithm. Ambiguous alignment areas were trimmed by Gblocks (Castresana 2000), the parameter ribosomal gene block with a minimum length was set to 2 base pairs (bp), and the allowed gap position was selected with half; the minimum length of the protein-coding gene block was set to 3 bp, and the allowed gap position was also selected with half.

For the barcoding dataset, the COI sequence fragment length was 510 bp after alignment and trimming. For the three-gene dataset, COI, 16S, and 28S sequences were aligned and trimmed to lengths of 624 bp, 471 bp, and 751 bp, respectively. Sequences of the multi-gene dataset were concatenated using Phylosuite v1.2.3.

The three-gene dataset was analyzed with partition schemes based on the genes and codons. PartitionFinder (Lanfear et al. 2017) was used to select the models for Bayesian inference (BI) analyses. ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the maximum likelihood (ML) analysis models in IQ-TREE (Minh et al. 2020). The selection of best-fit models was based on the corrected Akaike Information Criterion (AICc). Substitution models assigned to each partition by PartitionFinder and ModelFinder are listed in Suppl. material 1.

Neighbor-joining clustering and phylogenetic analyses

We used an integrative approach that combined molecular and morphological analyses for species delimitation and diagnosis. Based on the COI dataset, the NJ tree was constructed using the uncorrected *p*-distance model in

Table 1. List of COI sequences used in this study.

Species	GenBank accession number
<i>Postolata guangxiensis</i> Dai et al., 2023	OP009379
<i>Postolata guangxiensis</i> Dai et al., 2023	OP009380
<i>Postolata guangxiensis</i> Dai et al., 2023	OP009381
<i>Postolata guangxiensis</i> Dai et al., 2023	OP009382
<i>Postolata guangxiensis</i> Dai et al., 2023	OP009383
<i>Postolata guangxiensis</i> Dai et al., 2023	OP009384
<i>Postolata guangxiensis</i> Dai et al., 2023	OP009385
<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 1*	PP713224
<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 2*	PP713225
<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 3*	PP713226
<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 4*	PP713227
<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 5*	PP713228
<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 6*	PP713229
<i>Obovalis omiensis</i> (Heimburg, 1884)	LC518997
<i>Sinosolenia carinata</i> (Heude, 1877)	MG742248
<i>Sinosolenia oleivora</i> (Heude, 1877)	MG742249
<i>Ptychorhynchus pfisteri</i> (Heude, 1874)	MG742247
<i>Gonidea angulata</i> (Lea, 1838)	DQ272372
<i>Leguminaia anatolica</i> Gürlek et al., 2021	MZ511008
<i>Leguminaia saulcyi</i> (Bourguignat, 1852)	MZ510997
<i>Inversidens rentianensis</i> Wu & Wu, 2021	OR826138
<i>Lamprotula caveata</i> (Heude, 1877)	KJ434503
<i>Lamprotula leaii</i> (Gray, 1833)	MF072503

*Sequences from this study.

MEGA 7.0 (Kumar et al. 2016) with 1000 bootstrap replicates. Intraspecific and interspecific genetic distances were calculated based on the COI barcoding dataset using the uncorrected *p*-distance model in MEGA 7.0.

For the multi-locus dataset, the IQ-TREE web server (<http://iqtree.cibiv.univie.ac.at/>) performed maximum likelihood (ML) phylogenetic analysis using the ultrafast bootstrapping algorithm with 1000 repetitions. Bayesian inference (BI) phylogenetic analyses were carried out in MrBayes v2.01 (Ronquist et al. 2012) with generated models in PartitionFinder (Lanfear et al. 2017). Four independent Markov Chain Monte Carlo (MCMC) models were run simultaneously for ten million generations, and sampling was conducted every 1000 generations with a burn-in of 25%. The process terminated when the average standard deviation of the splitting frequency fell below 0.01. Ultimately, the constructed phylogenetic trees were implemented in the online iTOL (<https://itol.embl.de/itol.cgi>) to realize editing and visualization (Letunic and Bork 2007).

Results

Systematics

Family Unionidae Rafinesque, 1820
Subfamily Gonideinae Ortmann, 1916
Tribe Gonideini Ortmann, 1916

Genus *Postolata* Dai, Huang, Guo & Wu, 2023

Type species. *Postolata guangxiensis* Dai et al., 2023.

Table 2. Sequences from the three-gene dataset used for molecular analyses and corresponding GenBank numbers.

Family	Subfamily	Tribe	Taxa	COI	16S	28S
Unionidae	Gonideinae	Gonideini	<i>Ptychorhynchus pfisteri</i> (Heude, 1874)	MG463034	KY067440	MG595562
			<i>Obovalis omiensis</i> (Heimburg, 1884)	LC518995	LC223994	LC519064
			<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 1*	PP713224	PP717959	PP717965
			<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 2*	PP713225	PP717960	PP717966
			<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 3*	PP713226	PP717961	PP717967
			<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 4*	PP713227	PP717962	PP717968
			<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 5*	PP713228	PP717963	PP717969
			<i>Postolata longjiangensis</i> Liu & Wu, sp. nov. 6*	PP713229	PP717964	PP717970
			<i>Postolata guangxiensis</i> Dai et al., 2023 1	OP009379	OP020466	OP020470
			<i>Postolata guangxiensis</i> Dai et al., 2023 2	OP009380	OP020467	OP020470
			<i>Postolata guangxiensis</i> Dai et al., 2023 3	OP009381	OP020468	OP020470
			<i>Postolata guangxiensis</i> Dai et al., 2023 4	OP009382	OP020469	OP020471
			<i>Postolata guangxiensis</i> Dai et al., 2023 5	OP009383	OP020467	OP020472
			<i>Postolata guangxiensis</i> Dai et al., 2023 6	OP009384	OP020468	OP020470
			<i>Postolata guangxiensis</i> Dai et al., 2023 7	OP009385	OP020469	OP020471
			<i>Parvasolenia rivularis</i> (Heude, 1877)	KX966393	KX966393	MG595632
			<i>Koreosolenia sitgyensis</i> Lee et al., 2020	GQ451872	GQ451859	MT020817
			<i>Sinosolenia carinata</i> (Heude, 1877)	KX822669	MK683025	KX822626
			<i>Gonidea angulata</i> (Lea, 1838)	DQ272371	KF011258	AF400691
			<i>Microcondylaea bonellii</i> (Férussac, 1827)	KX822652	KP218021	KX822609
		Pseudodontini	<i>Bineurus loeiensis</i> Konopleva et al., 2021	KX865879	KX865650	KX865750
			<i>Bineurus anodontinum</i> (Rochebrune, 1882)	MW603662	MZ684076	MZ684018
			<i>Thaiconcha callifera</i> (Martens, 1860)	KX865862	KX865633	KX865734
			<i>Pseudodon mekongi</i> (Bolotov et al., 2020)	KX865861	KX865632	KX865733
			<i>Pseudodon vondembuschianus</i> (Lea, 1840)	KP795029	KP795052	MZ684028
			<i>Pilsbryoconcha exilis</i> (Lea, 1838)	KX051291	KX865646	KX822613
			<i>Indopseudodon kayinensis</i> (Bolotov et al., 2020)	MZ678754	MZ684081	MZ684033
			<i>Indopseudodon bogani</i> (Bolotov et al., 2017)	MF352218	MF352292	MF352350
			<i>Schepmania</i> sp. 5973	MZ678755	MZ684082	MZ684035
			<i>Lamprotula caveata</i> (Heude, 1877)	KX822646	NC_030336	KX822603
		Lamprotulini	<i>Lamprotula leaii</i> (Gray, 1833)	NC_023346	NC_023346	MG595524
			<i>Potomida littoralis</i> (Cuvier, 1798)	JN243905	NC_030073	JN243883
		Contradentini	<i>Yaukthwa inlenensis</i> Konopleva et al., 2019	KX865927	KX865681	KX865798
			<i>Yaukthwa paiensis</i> Konopleva et al., 2019	MH345972	MH346012	MH345992
			<i>Yaukthwa elongatula</i> Bolotov et al., 2019	MK372408	MK372456	MK372486
			<i>Lens contradens</i> (Lea, 1838)	MG581991	MT993693	MT993745
			<i>Lens eximius</i> (Lea, 1856)	KX865941	KX865689	KX865812
			<i>Physunio superbus</i> (Lea, 1843)	MG582020	MT993689	MT993741
			<i>Trapezoideus foliaceus</i> (Gould, 1843)	MH345985	MH346025	MH346005
		Rectidentini	<i>Hyriopsis bialata</i> Simpson, 1900	KX051274	MT993644	MT993697
			<i>Hyriopsis desowitzi</i> Brandt, 1974	KX822644	MT993679	KX822601
			<i>Rectidens sumatrensis</i> (Dunker, 1852)	KX051314	MW242818	KX822620
			<i>Ensidens sagittarius</i> (Lea, 1856)	KX865950	KX865696	KX865821
		Ctenodesmini	<i>Khairuloconcha lunbawangorum</i> Zieritz et al., 2021	MN900790	MZ684078	MN902294
			<i>Khairuloconcha sahanae</i> Zieritz et al., 2021	MZ678752	MZ684079	MZ684024
		Chamberlainiini	<i>Chamberlainia somsakpanhai</i> Kongim et al., 2023	KX822635	MK994770	KX822592
Margaritiferidae			<i>Margaritifera dahurica</i> (Middendorff, 1850)	KJ161516	KJ943526	KT343747
			<i>Margaritifera margaritifera</i> (Linnaeus, 1758)	KX550089	KX550091	KX550093

*Sequences from this study.

***Postolata longjiangensis* Liu & Wu, sp. nov.**

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Fig. 2

Type materials. *Holotype* (Fig. 2E): SXNU_24040702 (length 53.06 mm, height 27.11 mm, width 17.28 mm); Long River, Hechi City, Guangxi Province, China. *Paratypes* (Fig. 2A–D, F): five specimens, SXNU_24040703, SXNU_24040705, SXNU_24040706, SXNU_24040704, and SXNU_24040701. Same collection location as the holotype.

Morphological diagnosis. *Postolata longjiangensis* sp. nov. can be distinguished from *Postolata guangxiensis* by the shell shape, beak position, surface sculpture, nacre color, and hinge structure (Table 3). Diagnostic characteristics: shell elongated, irregularly rectangular; the umbo situated at 1/4 of the shell length; epidermis brown with greenish tinge; nacre blue-white; and hinge tooth weaker than that of *Postolata guangxiensis*.

Molecular diagnosis. *Postolata longjiangensis* sp. nov. and *Postolata guangxiensis* formed a closely related group within the tribe Gonideini. The sequences of

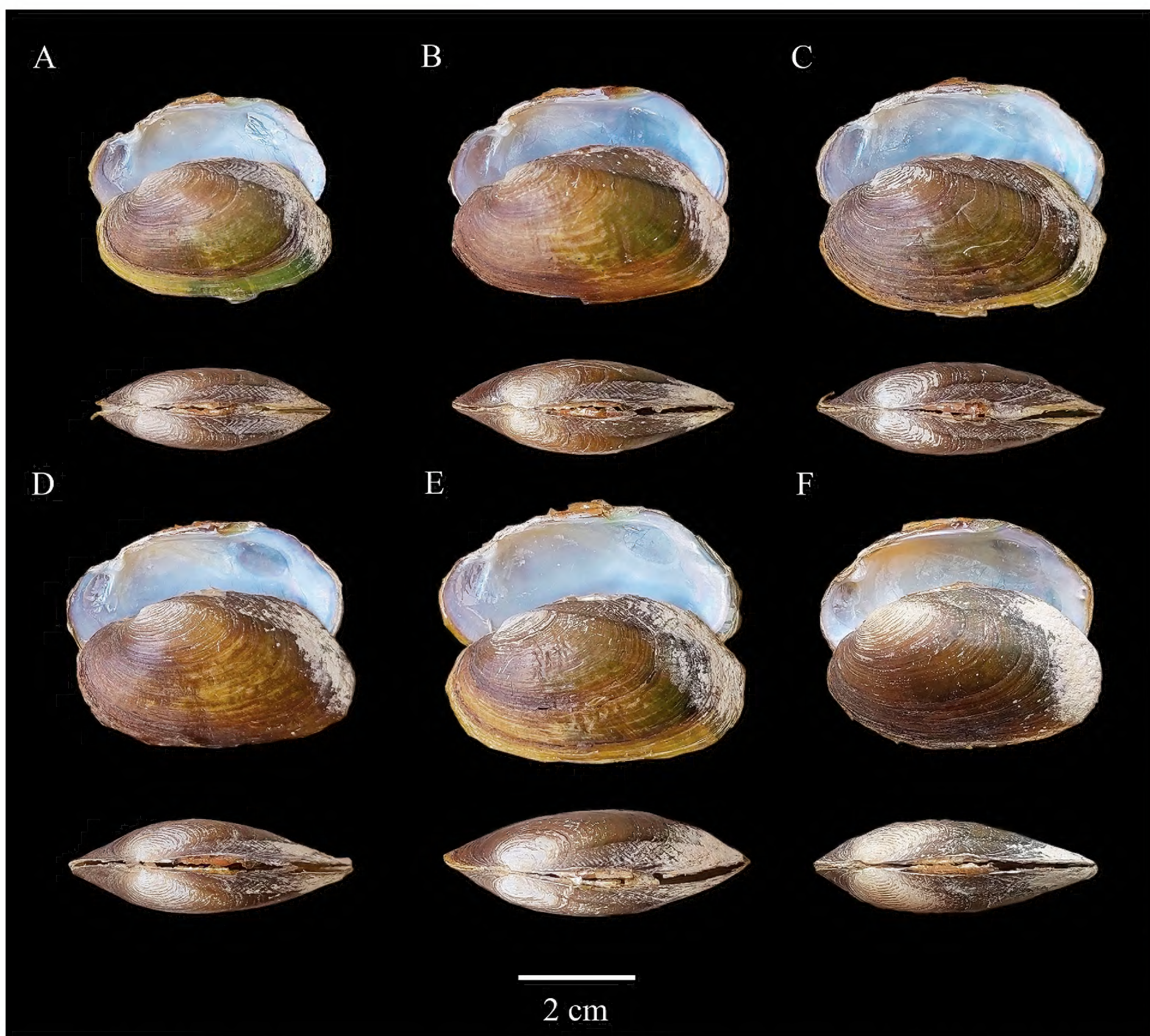


Figure 2. Shells of *Postolata longjiangensis* sp. nov. **A.** Paratype: SXNU_24040703; **B.** paratype: SXNU_24040705; **C.** Paratype: SXNU_24040706; **D.** Paratype: SXNU_24040704; **E.** Holotype: SXNU_24040702; and **F.** Paratype: SXNU_24040701.

Postolata longjiangensis sp. nov. revealed a well-supported lineage that is distinct from its congener (Fig. 4). The genetic distance between the new species and *Postolata guangxiensis* is 8.13% based on the COI barcoding sequences.

Description. Shell elongated, irregularly rectangular, slightly thickened, moderately inflated; anterior margin rounded and short; ventral margin nearly straight; posterior margin wide and long; posterior slope significantly prominent; dorsal margin nearly straight, with an upward tilt angle; umbo located at 1/4 of the shell length and sculptured with wavy ridges; epidermis brown with greenish tinge; shell surface sculptured with fine concentric growth lines (Fig. 2; Table 3). Anterior adductor muscle attachment oblong, little deep, and smooth; posterior adductor muscle attachment round to oval, shallow, and smooth; anterior retractor muscle attachment completely integrated with anterior adductor muscle attachment; posterior retractor muscle attachment irregularly round and fused with the posterior adductor muscle

attachment; mantle muscle attachment obvious. Hinge weakly developed; anterior tooth extremely small, posterior tooth small, thin, and pyramidal in the left valve; anterior tooth upright pyramidal; posterior tooth degenerate and merge into the lateral teeth in the right valve; there is one short lateral tooth of both shells; nacre-bule-white (Fig. 2; Table 3). Papillae in the incurrent aperture short and cylindrical, arranged in two rows; papillae in the excurrent aperture weakly developed, sparsely arranged in one row; and the pigmentation of the incurrent and excurrent aperture significant; the size of inner gills exceeds that of outer gills; labial palps medium-thick, flat elliptical (Fig. 3; Table 3).

Etymology. This species' name is dedicated to its collection location, the Long River in Hechi City, Guangxi Province, China. For the common name, we recommend “Longjiang Rear-wide Mussel” (English) and “Long Jiang Hou Ju Bang” (龙江后矩蚌) (Chinese).

Distribution. Long River at Hechi City, Guangxi Province, China.

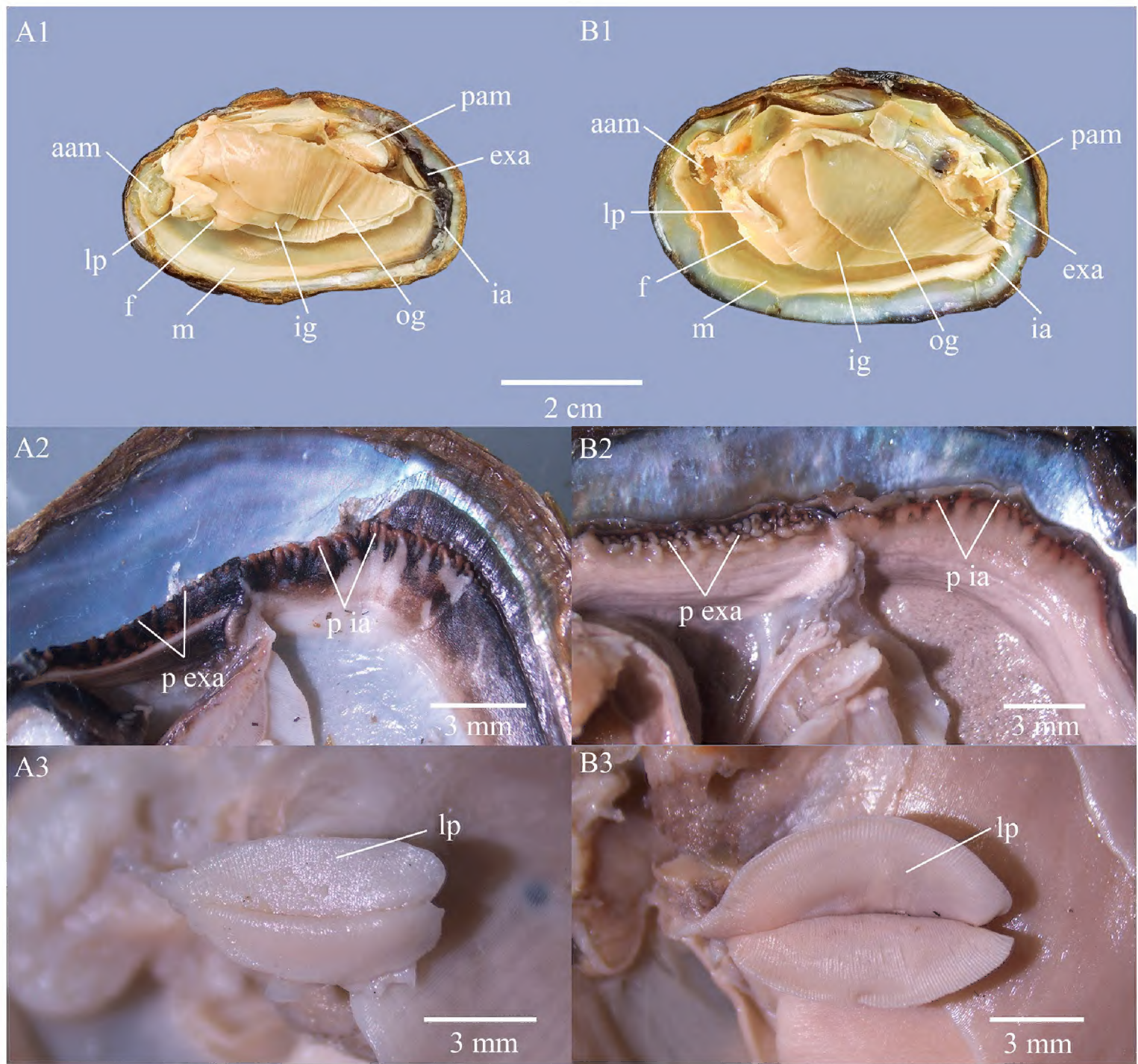


Figure 3. Anatomical features of *Postolata longjiangensis* sp. nov. and *Postolata guangxiensis*; **A1–3.** *Postolata longjiangensis* sp. nov.; **B1–3.** *Postolata guangxiensis*. Abbreviations: aam, anterior adductor muscle; pam, posterior adductor muscle; exa, excurrent aperture; ia, incurrent aperture; f, foot; ig, inner gill; og, outer gill; lp, labial palps; m, mantle; p ia, papillae in incurrent aperture; p exa, papillae of excurrent aperture.

Table 3. Conchological and soft-body characteristics of *Postolata longjiangensis* sp. nov. and *Postolata guangxiensis*.

Features	<i>Postolata longjiangensis</i> sp. nov.	<i>Postolata guangxiensis</i>
Length (mm)	41.14–49.93	49.22–57.76
Width (mm)	13.82–17.28	19.95–21.42
Height (mm)	22.89–27.63	33.47–39.34
Shell shape	Elongated, irregularly rectangular	Irregularly rectangular
Shell thickness	Slightly thick	Moderately thick
Umbo position and sculpture	1/4 of shell length; umbo sculptured with wavy ridges	1/3 of shell length; umbo often eroded
Surface sculpture	Epidermis is brown with a bit green; shell surface sculptured with fine concentric growth lines	Epidermis is black-brown; shell surface sculptured with fine concentric growth lines; there is one sulcus near the posterior dorsal margin
Nacre colour	Blue-white	Milky-white
Posterior slope	Significantly prominent	Insignificant
Dorsal margin	Nearly straight, with an upward tilt angle	Slightly curved downwards
Hinge	Weakly developed	Well developed
Pseudocardinal teeth of the left valve	Anterior tooth extremely small, posterior tooth small, thin, and pyramidal	Anterior tooth small, posterior tooth thick and pyramidal
Pseudocardinal teeth of the right valve	Anterior tooth upright pyramidal, posterior tooth degenerate and merge into the lateral teeth	Anterior tooth well-developed, posterior tooth reduced
Lateral teeth	One tooth on both valves, nearly straight	One tooth on both valves, small and short
Incurrent aperture	Papillae is short cylindrical, arranged in two rows; and pigmentation is significant	Papillae is distinctly short cylindrical, arranged in one to two rows
Excurrent aperture	Papillae is weakly developed, sparsely arranged in one row; and pigmentation is significant	Papillae is short and dense; pigmentation unnoticeable
Labial palps	Medium-thick, flat elliptical	Medium-thick, elliptical

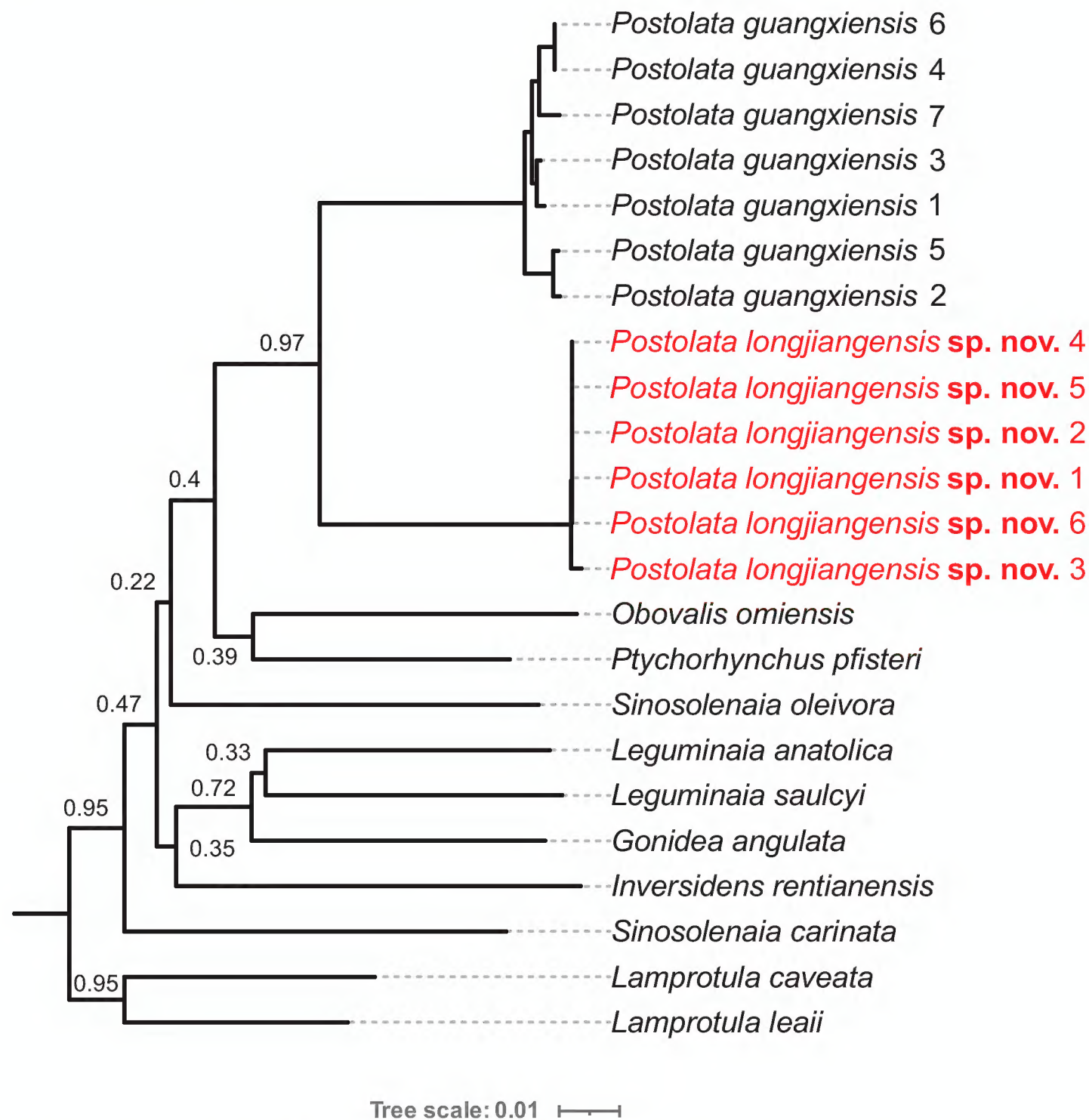


Figure 4. Neighbor-joining tree generated from 23 COI sequences (12 taxa) based on the uncorrected *p*-distance model. The numbers at the nodes indicate bootstrap supports (BS). The red fonts represent the species defined in this study.

Phylogenetic analyses

Multilocus phylogenies that were reconstructed using Bayesian inference (BI) and maximum likelihood (ML) analyses produced consistent topologies (Fig. 5). Both BI and ML analyses indicated that *Postolata longjiangensis* sp. nov. formed the sister lineage to *Postolata guangxiensis* in the tribe Gonideini with high support values (BS/PP = 96/0.96) (Fig. 5).

All eight recognized tribes in the subfamily Gonideinae formed monophyletic groups with the following relationships: ((Gonideini + (((Contradentini + Rectidentini) + Ctenodesmini) + (Lamprotulini + Chamberlainiini)) + (Pseudodontini + Schepmaniini)) (Fig. 5).

Discussion

We integrated comprehensive molecular evidence, shell morphology, and soft-body anatomy into the identification and classification of the new species from Guangxi, namely *Postolata longjiangensis* sp. nov. The topology of our phylogenetic tree (Fig. 5) basically corresponds

to those generated in previous studies, except for some nodes (Wu et al. 2024). In our phylogenetic tree, the six individuals of *P. longjiangensis* occupy a single branch that shares a sister-group relationship with *Postolata guangxiensis* in the tribe Gonideini (Fig. 5). The long branch of *P. longjiangensis* unequivocally indicates its distinct (species-level) divergence from congeneric species (uncorrected *p*-distance = 8.13%; Fig. 4).

In addition to the molecular phylogenetic evidence, *Postolata longjiangensis* and *Postolata guangxiensis* also display significant disparities in both shell morphology and soft-body anatomy (Table 3). The shell thickness of *P. longjiangensis* is relatively lower compared to that of *P. guangxiensis*, and the hinge is weakly developed, with only one prominent pseudocardinal tooth. The morphological characteristics of the apertures and labial palps also differ significantly (Fig. 3). The convergence of shell and anatomical features in freshwater mussels is an important factor contributing to the difficulty in species definition (Inoue et al. 2013; Lopes-Lima et al. 2024). However, both species of *Postolata* possess inter-specific diagnostic features in terms of both shell morphology and anatomy.

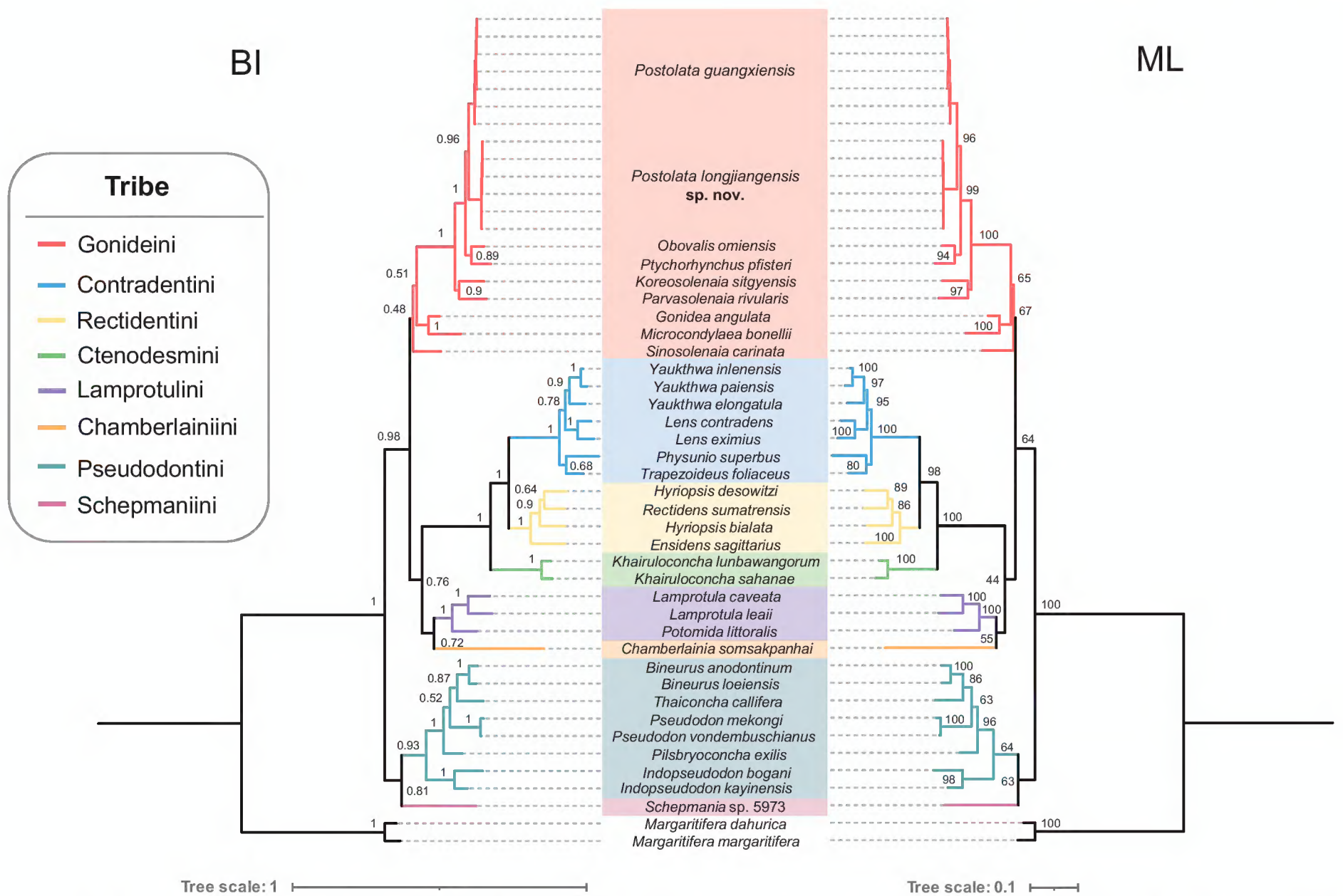


Figure 5. Bayesian inference (BI) and maximum likelihood (ML) trees reconstructed from the three-gene dataset (COI + 16S rRNA + 28S rRNA). Numbers at the nodes indicate the statistical support values for posterior probability (PP) and bootstrap support (BS). Color-coded clades and shadows represent eight tribes in the subfamily Gonideinae.

The southern region of Guangxi, situated in the Indo-Burma hotspot area, has garnered significant attention and conservation efforts for its rich biodiversity (Tordoff et al. 2012). However, there remains a dearth of research on freshwater mussels in this area, including accurate species distribution, precise classification, and population dynamics (Zieritz et al. 2018; Liu et al. 2022). This knowledge gap severely impedes the progress of mussel conservation in this region. The construction and planning of hydraulic projects for large rivers in recent years have led to the emergence of small rivers and tributaries as vital habitats for aquatic life (Jiang et al. 2011; Sabo et al. 2012; Xie 2017). The type locality of *Postolata guangxiensis* is a small tributary of the Luoqing River, characterized by good water quality and a silt bottom, with numerous residential structures in close proximity (Dai et al. 2023). It is interesting that *Postolata longjiangensis* was discovered 100 km away in the Long River and shares a similar habitat type (Fig. 1). The river habitats housing endemic mussel species are highly vulnerable and require immediate attention and protection due to the impacts of urbanization and human activities. Therefore, we advocate for the implementation of in situ conservation measures for select endemic and endangered freshwater mussels through the establishment of nature reserves. Additionally, comprehensive research on artificial breeding techniques and their practical application

is imperative to facilitate the recovery of this critically endangered species.

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Supplementary material 1

Partitioning schemes and best-fit models identified from PartitionFinder and ModelFinder for three-locus dataset

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Data type: xlsx

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